



# Classification of temperature response in germination of Brassicas<sup>☆</sup>

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## ABSTRACT

Since soil temperature affects germination and emergence of *Brassica napus* L., mustard [*B. juncea* (L.) Czerniak. and *Sinapsis alba* L.], and Camelina [*Camelina sativa* (L.) Crantz.] planting dates have to be adjusted to prevent crop failures. These crops can be used as biofuel feedstocks and some mustard varieties can be used as a soil biofumigant. Knowledge of germination temperature optima/range for brassicas is critical for inclusion of these crops into crop rotations. The *B. napus* varieties 'Clearwater' (UI-C-1), 'DKW 13-86' (Roundup® ready), and 'Gem' (UI-G-1); the mustards 'Caliente 61', 'Florida Broadleaf', 'Idagold', 'Kodiak Brown', and 'Pacific Gold', and the Camelina line 'NEB C-1' were germinated in the dark in Petri-dishes at 4, 10, 16, 21, 27 or 32 °C for up to 12 days. The shortest time to maximum germination (2 days) for all but 'Florida Broadleaf' and 'Kodiak Brown' occurred at 16 °C. 'NEB C-1' had the greatest percent germination at all temperatures except 32 °C where it was lower; 'DKW 13-86' decreased with increased temperature; 'Clearwater', 'Gem', 'Pacific Gold' and 'Kodiak Brown' had optimum germination between temperature extremes; 'Caliente 61' had the lowest maximum percent germination at 4 °C; 'Florida Broadleaf' had increased germination with increased temperature, and germination of 'Idagold' was not affected by temperature. Variability in Brassica seed germination in response to temperature indicates that there may be the opportunity for flexibility in the planting dates over which these crops can be sown.

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## 1. Introduction

The Brassicas are a group of plants that provide food, fodder and forage (Dixon, 2007). Increased interest in energy self-sustainability in the United States has brought new attention to the importance of the Brassicaceae including *Brassica napus* L., mustard (*Brassica* sp. and *Sinapsis* sp.) and camelina [*Camelina sativa* (L.) Crantz.] (Putnam et al., 1993) as biofuel feedstocks. In the United States production of Brassicas for biofuel has until recently been primarily in the northern tier of states. In that portion of the US, and adjacent areas of Canada, the concern is how to best germinate seed at temperatures from 10 to 22 °C (Nykiforuk and Johnson-

Flanagan, 1994; Zheng et al., 1994; Vigil et al., 1997; Willenborg et al., 2004). Brassica production is being considered for other geographic areas in the United States where higher soil temperatures could occur at sowing. Varieties of winter hardy *B. napus* have been developed for use in the southern Great Plains with establishment dates from mid-August to late-September for harvest the following spring (Boyle et al., 2004a,b). Soil temperatures range from 23 to 28 °C. Russo and Bruton (2008) reported that planting dates for *B. napus* in Oklahoma could be extended to late-October for some varieties; soil temperatures can be 20 °C at this time.

In the southern Great Plains, an alternative to over-wintering *B. napus* could be establishment in late-spring for a possible harvest in early-fall, but there may be problems in obtaining acceptable seed yields. Specifically, this may be due to flowering during times when temperatures do not favor athesis which was reported to be about 20 °C (Angardi et al., 1999, 2000; Nuttall et al., 1992). However, this has not been definitively established for the southern Great Plains. Some members of the Brassicaceae contain compounds implicated in reduction of soilborne pathogens, insects, and weed seeds (Charron and Sams, 1999; Noble et al., 2002; Wiggins and Kinkel, 2005). Varieties of mustard [*B. juncea* (L.) Czerniak.] produce isothiocyanate compounds which volatilize following injury to the plant. To introduce isothiocyanate compounds to the soil biota, the green biomass must be disrupted and/or chopped and quickly turned into the soil to achieve maximum concentration and

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**Table 1**

Sources of seed and seed descriptions.

Source	Variety	Type	Binomial	Seed color	Season length
High Point Seeds <sup>a</sup>	Caliente 61	Mustard	<i>B. juncea</i>	Yellow	Long <sup>b</sup>
University of Idaho	Pacific Gold	Mustard	<i>B. juncea</i>	Yellow	Long
University of Idaho	Idagold	Mustard	<i>S. alba</i>	Yellow	Long
Mayo seed	Florida Broadleaf	Mustard	<i>B. juncea</i>	Brown	Long
High Point Seeds	Kodiak Brown	Mustard	<i>B. juncea</i>	Brown	Short
University of Idaho	NEB C-1	Camelina	<i>C. sativa</i>	Orange	Short
Monsanto	DKW13-86	— <sup>c</sup>	<i>B. napus</i>	Brown	Long
University of Idaho	Clearwater	—	<i>B. napus</i>	Brown	Short
High Point Seeds	Gem	Oil seed rape	<i>B. napus</i> <sup>d</sup>	Brown	Short

<sup>a</sup> High Point Seeds (Dale Gies), Moses Lake, Washington; Mayo Seed Co., Knoxville, TN; Monsanto, St. Louis, MO; Univ. of Idaho, Moscow, ID.<sup>b</sup> Season length is based on time to seed harvest; long in this context means the plant is established in the fall for harvest the following year, short in this context means that establishment and harvest occurs in the same year. The dual use of mustard as a biofumigant would change the definition of season length since the aim would be production of biomass not seed.<sup>c</sup> Type name not used.<sup>d</sup> *B. napus* and rape are in the same genus species; usage, forage or oilseed, to some degree defines classification (Dixon, 2007).

exposure. The soil fumigant methyl bromide, used in the United States, was completely phased out in 2005, although critical use exemptions can be issued (USEPA, 2007). As a result, producers have limited options for controlling soilborne pathogens. Mustards, used as a biofumigation crop, may provide an alternative to the use of methyl bromide. Mustards can be used as an oil seed, and as a biofuel feedstock (Dixon, 2007). Before the crop can be incorporated into existing crop rotations in southern states, variables affecting planting date, plant development, and seed harvest must be determined.

Although many Brassicas are considered cool-season crops, air temperature may be more important in setting flowers and fruit (Dixon, 2007). Also important to know is the range of temperatures over which seed of these crops will germinate. Both criteria are important in determining how to integrate these crops into rotations. For cultivation in the southern states, it is especially important to determine how high soil temperature affects germination. The long growing season in the southern Great Plains could allow for flexible planting dates that could produce seed yield for canola, Camelina and mustards, or biomass yield for mustard used in biofumigation. This project was undertaken to determine how temperature affects time to germination and total germination of selected Brassica varieties.

## 2. Materials and methods

Twenty-seed of each variety of *B. napus* or mustard [*B. juncea* and *Sinapsis alba* L.] and the camelina (*C. sativa*) line (Table 1) were placed in individual Petri-dishes containing two layers of Whatman 42 filter paper (Fisher Scientific, Houston, TX). The filter paper was moistened with 20 mL of distilled water and Petri-dish covers put in place. Seeds were incubated in the dark in walk-in chambers (Artic Temp, Meeker, OK) at air temperatures of 4, 10, 16, 21, 27 or 32 °C with a range of  $\pm 0.25$  °C for up to 12 days. The same chamber was used for the same temperature for each replication. The entire experiment was replicated three times with 5 Petri-dishes in each replication (100 seed per replication), for a total of 15 Petri-dishes for each variety or line at each temperature. Seed were from the same lot of each variety or line.

Seed were checked daily and days to total germination and effect of temperature on germination determined. When the paper in any Petri-dish began to dry at the edges an additional 10 mL of distilled water was added to Petri-dishes. Seed were considered to be germinated when the radicle was at least twice the length of the seed.

Data were subjected to analysis of variance and it was determined if the data fit any linear distribution. If data did not fit linear distributions analysis was with PROC GLM in SAS (SAS Inc., Cary,

NC) to determine significance of main effects and their interaction. If an interaction was present it was used to explain results and means were separated with Least Squares Means analysis.

## 3. Results

Seed exhibited synchronicity in germination. Maximum germination occurred within 3 days of the start of germination regardless of temperature. Maximum germination did not always represent 100%, 80%, or better, germination was deemed to be acceptable. It was considered beneficial if time to obtain 80% germination was less than 4 days. Ungerminated seed were not empty. The reason for non-germination of those seed is not immediately clear.

The data did not fit linear distributions. ANOVA analysis indicated that only temperature affected days to maximum germination ( $P \leq 0.01$ ) and percent maximum germination ( $P \leq 0.05$ ) for the Camelina line. ANOVA indicated that for *B. napus* temperature ( $P \leq 0.01$ ), variety ( $P \leq 0.05$ ) and their interaction ( $P \leq 0.01$ ) affected days to maximum germination and percent maximum germination; for the mustards temperature, variety and their interaction (all  $P \leq 0.01$ ) affected days to maximum germination and percent maximum germination.

### 3.1. Camelina

#### 3.1.1. Time to maximum germination and maximum germination percent over temperatures

For line 'NEB C-1' maximum germination at 16 °C, and higher, occurred by 2 days. Below 16 °C maximum germination occurred by 5 days at 10 °C and 9 days at 4 °C. At all temperatures but 32 °C, there was 100% germination; at 32 °C there was less than 80% total germination.

### 3.2. B. napus

#### 3.2.1. Time to maximum germination and maximum germination percent over temperatures

The patterns for days to germination and percent maximum germination varied for varieties of *B. napus* (Table 2). The varieties 'DKW 13-86', 'Clearwater' and 'Gem' had longer times to maximum germination at 4 °C; fewer days at 10 °C, and the shortest time to maximum germination at 16 °C and above. For 'DKW 13-86' maximum percent germination was similar and highest from 4 to 16 °C. Maximum percent germination was lower through 32 °C. For 'Clearwater' maximum germination occurred at 10 °C and then decreased, and stabilized, from 16 °C on. For 'Gem' maximum germination increased through 21 °C and was unchanged through 32 °C.

**Table 2**Interaction of temperature and variety on time to maximum germination and maximum germination percent for *B. napus* and mustard varieties separately.

Variety	Temperature	Days to max. germination	Maximum % germination
<i>B. napus</i>			
DKW 13-86	4	9	94
	10	5*	96 <sup>NS</sup>
	16	2*	94 <sup>NS</sup>
	21	2 <sup>NS</sup>	83*
	27	2 <sup>NS</sup>	82 <sup>NS</sup>
	32	2 <sup>NS</sup>	68**
Clearwater	4	12	85
	10	9*	98**
	16	2**	93*
	21	2 <sup>NS</sup>	92 <sup>NS</sup>
	27	2 <sup>NS</sup>	92 <sup>NS</sup>
	32	2 <sup>NS</sup>	92 <sup>NS</sup>
Gem	4	12	31
	10	5**	82**
	16	2*	84 <sup>NS</sup>
	21	2 <sup>NS</sup>	90*
	27	2 <sup>NS</sup>	91 <sup>NS</sup>
	32	2 <sup>NS</sup>	92 <sup>NS</sup>
Mustards			
Florida Broadleaf	4	12	45
	10	12	88**
	16	5**	90 <sup>NS</sup>
	21	2*	97*
	27	2 <sup>NS</sup>	99 <sup>NS</sup>
	32	2 <sup>NS</sup>	100 <sup>NS</sup>
Pacific Gold	4	12	70
	10	12 <sup>NS</sup>	70 <sup>NS</sup>
	16	5**	92**
	21	2*	96 <sup>NS</sup>
	27	2 <sup>NS</sup>	94 <sup>NS</sup>
	32	2 <sup>NS</sup>	83*
Kodiak Brown	4	9	71
	10	9 <sup>NS</sup>	70 <sup>NS</sup>
	16	2*	96**
	21	2 <sup>NS</sup>	97 <sup>NS</sup>
	27	2 <sup>NS</sup>	95 <sup>NS</sup>
	32	2 <sup>NS</sup>	76**
Caliente 61	4	9	88
	10	7*	99**
	16	2*	97 <sup>NS</sup>
	21	2 <sup>NS</sup>	98 <sup>NS</sup>
	27	2 <sup>NS</sup>	97 <sup>NS</sup>
	32	2 <sup>NS</sup>	95 <sup>NS</sup>
Idagold	4	12	100
	10	12 <sup>NS</sup>	100 <sup>NS</sup>
	16	2*	98 <sup>NS</sup>
	21	2 <sup>NS</sup>	98 <sup>NS</sup>
	27	2 <sup>NS</sup>	98 <sup>NS</sup>
	32	2 <sup>NS</sup>	93 <sup>NS</sup>

<sup>NS</sup>, \*, \*\* non-significant or significant at  $P < 0.05$  or  $P < 0.01$ , Least Squares Means analysis. To explain the interaction of variety and temperature that occurred in each type of Brassica the analysis compares the affect of temperature within a variety, not the affect of analysis between varieties at a specific temperature. The abbreviations describing significance level indicate increase or decrease in days to germination and germination percentage that occurs as temperature increases.

### 3.3. Mustards

#### 3.3.1. Time to maximum germination and maximum germination percent over temperatures

The patterns for days to germination and percent maximum germination varied for mustard varieties (Table 2). In general, the longest times to germination for all varieties occurred at 4 and 10 °C. At higher temperatures maximum germination occurred by 2 days. The exception was for 'Pacific Gold' where the shortest times to reach maximum germination occurred at 21 °C or above.

Germination for 'Florida Broadleaf' increased with increasing temperature through 21 °C and stabilized at higher temperatures. For 'Pacific Gold' and 'Kodiak Brown' germination at 4 and 10 °C

was similar and lower than at 16–27 °C; germination decreased at 32 °C. Germination for 'Caliente 61' was lowest at 4 °C; germination at all other temperatures was similar. Temperature did not affect germination of 'Idagold'.

## 4. Discussion

Seed of the various Brassicas exhibited differences in temperature requirements for germination which could influence planting date and adequate stand establishment. Some mustard varieties can be used as a biofumigant (Charron and Sams, 1999; Noble et al., 2002). If used for that purpose, effects that temperature has

on seed yield would not be an important consideration as long as biomass yields are adequate.

Optimum temperatures for germination were based on a minimum of 80+% germination in <5 days. Time to germination and emergence often dictates overall crop performance. As an example, it took 12 days to achieve 100% germination at 4 °C for 'Idagold' which is probably not acceptable. It is apparent that all varieties tested did not have the same germination potential at a given temperature. Patterns of germination could be classified as: decreasing with increasing temperature, having an optimum temperature between extremes, increasing with increasing temperature, or not affected by temperature. This may allow specific Brassicas to be matched with specific planting dates at different times of the year and particular regions based on soil and/or air temperatures.

Crops that are generally grown in cooler climates may not be adapted to the same planting windows if established in other climates. Designations of season length may lose meaning in different climates and the same crops may do well if established for harvest in other planting windows. In addition if mustard is grown for biomass rather than seed, the terminology of season length must be adjusted. Mustards may be able to be established in late-winter in warmer climates and produce sufficient biomass before being turned under to act as a biofumigant for spring planted crops. It may also be possible to sow various Brassicas in early spring in warm climates so that seed can be harvested and other crops follow in rotation before it is necessary to re-establish a winter cover. How temperature affects germination is an important consideration in these decisions. Germination responses for the Brassicas tested can be considered in relation to soil temperatures at various times of the year in different locations to identify possible planting windows appropriate to specific crops.

## 5. Conclusion

Temperature affected time to germination and total germination. Crop types, and varieties within crops, responded differently to temperature with some being more responsive to warmer temperatures and others being able to germinate across a wide range of temperatures. Another consideration for study is whether seed oil content or quality affects germination. Additional testing is

required to determine if germination responses under controlled conditions can be replicated under field conditions. The data can be used as a starting point to determine the varieties to use in field establishment studies across a wide variety of locations.

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